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RESEARCH ARTICLE

VECTORS IN PERIODONTAL GENE THERAPY– A REVIEW

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ABSTRACT

Gene therapy is fast becoming one of the most contentious subjects in contemporary medicine and science as technology continues to evolve exponentially. Periodontal disorders have a wide variety of inflammatory and damaging reactions and are believed to have a multi-factorial cause. Genetic variation was found to be a significant risk factor for periodontitis. Genetic methods in periodontal tissue engineering demonstrate early promise in supplying growth factor genes to periodontal lesions.

Key words: Gene Therapy, Periodontitis, Vector.

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INTRODUCTION

Gene therapy is a fieldofbiomedicine that requires removing or restoring faulty genes in the diseased cell genome to restore natural cell activity without triggering any toxic effects to non-target tissues. Since the first attempt at human gene therapy in 1980, scientists and physicians have been actively studying and performing experiments. Gene therapy is fast becoming one of the most contentious subjects in contemporary medicine and science as technology continues to evolve exponentially. Modern testing methods are becoming more popular, including gene injection and alteration into species such as plants and livestock, and society is becoming closer to adapting these methods to human therapy and treatment of illnesses. Periodontal diseases are persistent inflammatory diseases of the supportive tissues of the teeth triggered by particular microorganisms, culminating in the gradual degradation of periodontal ligaments, alveolar bones created by pockets, recesses, or both. It is multifactorial, consisting of a microbial threat and variable host immune response modified by genetic and environmental factors. Periodontitis is typically introduced as a response to bacteria colonizing the tooth surface and the gingival crevice. Most of the damage that happens in the host periodontal tissues is immune-mediated and inflammatory immune responses that are known to be a host protective mechanism to defend against infection.[1] Genetic methods in periodontal tissue engineering demonstrate early promise in supplying growth factor genes to periodontal lesions. Gene therapy approaches jointly interface and supplement stem cell therapy and new scaffolding technology to improve their ability to recover tissue function and form invariably. The Tissue Engineering Technique reconstructs the natural target tissue by integrating three components, including scaffolding, signaling molecules, and cells. The area of tissue engineering has entered a point where several usable tissues may be developed or regenerated in the laboratory or the patient. Periodontal tissue engineering comprises of three approaches. Protein-based, cell-based, or gene-based.[2] In this review, the gene-based approach is discussed.

Gene therapy approaches in Periodontitis[2]

In Vivo: The in-vivo technique for gene transfer includes the infusion or implantation of genetic material directly into the host. In-vivo gene therapy locally targets cells or tissue at the injection site. This method minimizes the possibility of infection, as only one treatment is needed for the patient. However, it is highly difficult to transfect in-vivo cells resulting in low levels of protein expression, and, importantly, it is difficult to prevent the expression of the growth factor in secondary tissues. To resolve some of the difficulties associated with direct injection distribution, which involve clearance from the delivery site and vector deterioration or

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inactivation, scaffolding should be used along with a bioactive signal. Cell and gene-based systems that utilize scaffolding matrices for periodontal tissue engineering.

- Extra oral and intraoral stem cells are a suitable and usable alternate source for harvesting and expansion of multipotent colonies. Adequate cell density could be achieved in vitro in a stable setting and made readily accessible for re-implantation at a periodontal defect location.
- The available direct and cell-based transmission of the therapeutic gene has been shown to improve the regenerative capacity and increase the abundance of essential factors. The gene of interest is either inserted directly into the periodontal defect by a retrovirus or it may be integrated into an embryonic stem cell (ES) or adult stem cell that is eventually enlarged and transported to the region of interest.
- Prefabricated and image-based scaffolds are becoming an integral component of regenerative medicine. A specified supporting framework enables the position and guidance of the related cells and proteins and the creation of a mechanically competent environment. Periodontal regeneration scaffolds are distributed in particulate, rigid, and inject table types. New technology has made it possible to customize scaffolds that match into a periodontal defect and have an external and an internal defect.

Ex vivo: The ex-vivo gene transfer approaches include the in-vitro genetic modification of cells and the reintroduction of cells into the host with or without scaffolding. The ex-vivo approach is, in many ways, better for the patient since the immune reaction to virus particles or the infectious and harmful consequences of transfection agents are reduced. The downside is that it requires two different intrusive treatments for the patient, which raises the possibility of infection. However, it is possible to select the form of transduced or transfected cells.

Vector: A gene that is incorporated directly into a cell does not normally function. Instead, a carrier called a vector is used to inject the therapeutic gene into the target cells of the patient. The most famous vector used is a virus that has been genetically engineered to bear regular human DNA. Viruses induce diseases of humans by encapsulating and transmitting genes to cells. Such forms of viruses, such as retroviruses, incorporate their genetic material (which can be modified to contain the therapeutic gene) into the human cell chromosome. Other viruses, such as adenoviruses, have their DNA incorporated into the nucleus of the cell, but the DNA is not absorbed into the chromosome. The vector may be administered intravenously or injected directly into a particular tissue in the body where it is picked up by individual cells (target cells). Alternatively, a sample of the patient's cells will be extracted and subjected to the vector in a laboratory setting; the vector-containing cells are then reintroduced into the patient.[2]

Four of the various forms of viruses used as vectors for gene therapy are:[3] Retroviruses (e.g. HIV): a family of viruses that may produce double-stranded copies of their DNA genomes. These copies of the genome may be inserted into host cell chromosomes.

- Adenoviruses: A family of double-stranded DNA genome viruses that cause gastrointestinal, intestinal, and eye infections in humans.
- Adeno-associated viruses: a class of small, single-stranded DNA viruses that can inject their genetic material at a particular chromosome 19 site.
- Herpes simplex viruses: a family of double-stranded viruses that can invade a specific form of cell, i.e. Neuron.

One of many methods can be used to fix defective genes:[4]

- A normal gene can be implanted into an unspecific position within the genome to substitute a non-functional gene; this is the most popular method.
- A defective gene may be substituted for a regular gene by homologous recombination.
- A mutated gene could be restored by selective reverse mutation, which restores the gene to its normal working status.
- The control (the degree to which the gene is activated on or off) of a single gene could be modified.

Gene therapy vectors used in periodontics are:

1. **Viral**

- Vectors of Adenovirus (AV)
- AAV (Adeno-Associated Viral Vector)
- Recombinant Adeno-Viral Vectors
- Lentivector

2. **Non-viral**

- Gene-Active Matrix (GAM)
- Bubble Liposome and Ultrasound for Gene Transmission

Adenovirus Vectors (AV): Adenovirus vectors are the most widely used vectors for gene therapy. They are also used as vaccinations for the expression of antigens. Adenovirus vectors can be replicative-defective; some important viral genes are removed and substituted by a cassette that expresses a foreign therapeutic gene.[5] Bone morphogenetic proteins (BMPs) have significant potential for periodontal tissue regeneration. Their limitations include the temporary biological function and their poor bioavailability at the wound site. Gene transfer may function as an alternate therapy method to provide BMPs to periodontal tissues.[6]Adsare highly immunogenic and substantial engineering is needed to avoid the leakage of the gene resulting in the removal of transduced cells by CTL in vivo.[7] Noggin is a BMP bioactivity blocker that binds to selected BMPs and inhibits the binding of BMP-2,-4, and-7 to cell surface receptors. Bone lesions treated with AV / BMP-7 gene delivery displayed accelerated chondrogenesis, accompanied by osteogenesis, cementogenesis, and predictable bridging of periodontal bone defects. The findings of this experiment showed the first successful proof of periodontal tissue engineering utilizing ex vivo BMP gene transfer.21[8] Adenoviral vectors for ex vivo BMP-7 were evaluated along with noggin gene transfer to promote tissue engineering in rats with large mandibular bone defects.[8]In a related sample, the impact of BMP-7 and noggin gene transfer in an extreme combined immunodeficiency (SCID) mice model was done.[6]

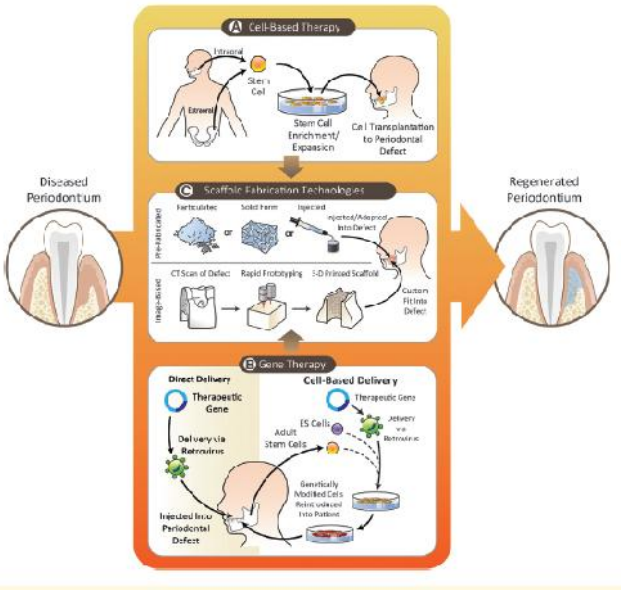

Photo courtesy: Rios HF, Lin Z, Oh B, Park CH, Giannobile WV. Cell-and gene-based therapeutic strategies for periodontal regenerative medicine. J Periodontol2011 ;82:1223-37

Another study tested periodontal attachment recovery in a rabbit sample utilizing mixture of ex vivo autologous bone marrow mesenchymal stem cells (MSCs) and AV for BMP-2 gene transmission.[10] In a related analysis, ex-vivo AV / BMP-2 gene transmission using canine periodontal ligament stem cells (PDLSCs) was evaluated for peri-implantitis defects in dogs. In conclusion, the ex vivo BMP2 gene distribution utilizing PDLSCs improved new bone structure and re-osseointegration in peri-implantitis defects.[11] The findings of this study have shown that AV / PDGF-B gene delivery has sustained signal transduction effects in human gingival fibroblasts. The functions of the hepatocyte growth factor (HGF) along with human dental pulp stem cells (DPSCs) in periodontal tissue regeneration have also recently been studied. Results suggest that adenovirus-mediated transfer of the hepatocyte growth factor gene to human dental pulp stem cells increased the capacity for periodontal regeneration in the swine model.[12]

AAV (Adeno-Associated Viral Vectors): Adeno-associated viral vectors are derived from a replication-deficient, non-pathogenic parvovirus with a single-stranded DNA genome and are especially successful in the transduction of non-dividing cells.[13] AAVs have been suggested as a gene transmission vector to inhibit the development of the periodontal disorder in rats. Cirelli *et al*. demonstrated the usage of serotype 1-based pseudotyped adeno-associated virus vector (AAV2/1) to deliver TNF receptor-immunoglobulin Fc (TNFR: Fc) to rats experiencing Porphyromonas gingivalis (P.g.). The findings of this analysis revealed that AAV2/1-TNFR: Fc contributed to potent inhibition of periodontal disease progression.[14] The AAV-mediated RNAi knockdown (sh) of Atp6i / TIRC7 gene expression was examined in the treatment of periodontal disease wherein Mice had been treated with P. gingivalis W50 in maxillary periodontium to cause periodontitis. Data indicate that AAV-shRNA-Atp6i / TIRC7 therapy could significantly improve the health of mice with periodontal disease-mediated P. gingivalis.[15] In a more recent in vivo experiment with mice, the function of cathepsin K (Ctsk) in chronic periodontal infection and inflammation was examined.

To this end, the animals were contaminated with P. gingivalis and the tiny hairpin (sh) RNA (AAV-sh-Ctsk) was used to silence cathepsin K. As a consequence, AAV-mediated Ctsk silencing successfully shielded animals from periodontal tissue injury and alveolar bone loss.[16]

rAds (Recombinant Adeno-Viral) Vectors: Wild adenoviruses, like human adenovirus type 5, are linked with a variety of minor illnesses, such as respiratory infections. Recombinant adeno-associated virus (rAds) is a purified replication of human parvovirus that enables it to be safely used as a gene delivery method. rAds have been used as gene distribution vectors owing to a variety of special features:[17]

- Ads have a strong transduction ability both in dividing and non-dividing cells;
- Ads do not cause apparent phenotypic shifts in transduced cells;
- Advertisements do not merge into the host genome and stay episomal.

The recombinant adenoviral vector (rAds) encoding for a platelet-derived growth factor: was examined foragene that delivers PDGF transgenes to cells to facilitate periodontal tissue regeneration. The results showed continuous transmission of PDGF genes inducedcementoblast activity in vitro. In conclusion, it was proposed that this treatment may provide the benefit of supplying in vivo recombinant proteins to tissues for prolonged periods as a new approach to periodontal tissue engineering.[18] In an in vitro assay, ex-vivo-transfection of rAds (Ad2) encoding PDGF-A or PDGF-1308 to periodontium-derived cells. As a consequence, it has been stated that Ad2 / PDGF effectively transduced periodontium-derived cells and stimulated biological activity. This research promotes the possible usage of gene therapy for the sustained release of PDGF in periodontal tissues.[19]

Lentivector: In recent years, the study has centered on the usage of lentitizers, which, like their basic retrovirus equivalents, are devoid of viral proteins, free from replication of a competent virus, and additionally capable of transducing non-dividing cells. This function is useful for many gene therapeutic applications targeting post-mitotic, strongly differentiated cells. These lentitives are currently used in approximately 1.4% of clinical trials.[20] Lentivector transfection can be used to examine the inhibition of osteogenic differentiation of human PDL cells (hPDLCs) by follicular dendritic cell secreted protein (FDC-SP). The findings of one clinical trial demonstrated that FDC-SP transfection had a negligible adverse effect on the proliferation of hPDLCs and implied the biological function of FDC-SP as a fibroblast phenotype stabilizer by inhibiting the differentiation of hPDLCs into mineralized tissue-forming cells, thus regulating periodontal tissue regeneration.[21] Lentivector vectors represent a potential for transducing many forms of non-dividing cells.[22] Lentivector gene delivery may be an appropriate strategy for alveolar bone repair applications requiring sustained, long-term expression of therapeutic proteins, but they also have some safety concerns.

Gene-activated matrix: Gene-activated matrix (GAM) technology is a direct gene transfer technique that incorporates the two strategies — tissue engineering and the local gene delivery mechanism for periodontal tissue regeneration. GAM acts as a local bioreactor of therapeutic gene expression and

offers a systemic template to resolve lesion defects for cell adhesion, proliferation, and extracellular matrix synthesis (ECM). As GAM is inserted into a tissue defect, the granulation tissue fibroblasts proliferate and migrate to GAM and pick up and transiently express the therapeutic gene to facilitate tissue regeneration.[23] While a variety of studies are based on the safety profile of adenovirus-mediated gene therapy, few of them have examined the local distribution of AVVs utilizing a gene-activated matrix. Chang *et al* examined the local administration of AVV encoding human PDGF-B with a gene-activated collagen matrix. The findings showed that the DNA vector-E1-, E3-deleted human adenovirus serotype 5 vectors are a healthy approach for delivering tooth-supporting alveolar bone defects. No treatment-related toxicity or systemic intervention has been established and these findings encourage further clinical advancement of AV / PDGF-B for oral and craniofacial bone regeneration therapy.[24]

Bubble Liposomes and Ultrasound for Gene Delivery: Bubble liposomes were formed as a valuable carrier for gene or drug distribution and recorded improved delivery performance with high-frequency ultrasound. The goal was therefore to investigate the possibilities of transmitting genes to gingival tissues utilizing bubble liposomes and ultrasound. Sugano *et al* contrasted the transmission of bare plasmid DNA encoding luciferase or enhanced green fluorescent protein (EGFP) to the lower labial gingiva of Wistar rats utilizing bubble liposomes, with or without ultrasound exposure. In conclusion, the combination of bubble liposomes and ultrasound offered an effective strategy for transporting plasmid DNA to the gingiva, and bubble liposomes may be considered a versatile carrier for the transmission of genes or medicines. This method can be used to provide several therapeutic molecules to the target tissue which can act as a viable treatment tool for periodontitis in the future.[25] The difficulty of the gene-enhanced periodontal regenerative therapy resides in the determination of which gene or gene combinations are required and adequate to promote the regeneration of several tissue types in the periodontium. In the future, it could be possible to replicate the natural healing mechanism by creating novel biomimetic scaffolds that respond to or release environmental stimuli (proteins or genes) according to individual cellular requirements. Cooperation between tissue engineering and periodontal practitioners can ultimately help to solve the challenges.[26]

Significant studies supporting Periodontal Gene Therapy

Established growth factor (PDGF) gene delivery:

- In their research, Jin *et al*.[9] demonstrated that direct in vivo gene transfer of PDGF-B induced tissue regeneration in large periodontal defects. Adenovirus-containing matrix encoding luciferase (control), a dominant-negative PDGF-A (PDGF-1308), or PDGF-B mutant were used as a vector. Results indicated that in vivo direct gene transfer of PDGF-B stimulates the regeneration of the alveolar bone and cementum in large periodontal defects. Gene therapy using PDGF-B may offer potential applications for periodontal tissue engineering.
- Anusaksathien *et al*.[27] in an ex vivo investigation found that the expression of PDGF-A and PDGF-B genes in gingival wounds has been extended for up to 10 days. The three-dimensional collagen lattices of Human Gingival Fibroblasts were transduced by adenovirus encoding successfully.
- Giannobile *et al*.[18] reviewed diverse drug delivery pathways and new approaches to oral and tooth-supporting reconstructive systems, including periodontium and alveolar bone. Recombinant adenoviral vectors encoding the PDGF-A gene have been constructed to facilitate the transmission of PDGF transgenes to the cells. These results indicated that the gene distribution of the platelet-derived growth factor activates cementoblast development that is maintained beyond that of the rhPDGF-AA application.

Delivery of bone morphogenetic protein:

- Franceschi *et al*.[8] explored the in vitro and in vivo transition of the BMP-7 gene to bone formation. The osteogenic effect of AdCMV-BMP7, an adenovirus containing BMP7 cDNA under the guidance of the CMV promoter was built using Cre / lox recombination. Data obtained demonstrated that the AdCMV-BMP7 transduced cells generated biologically active BMP-7 both in vitro and in vivo, and demonstrated that direct viral transduction gene therapy utilizing a virus/matrix implant may be a feasible route to inducing bone regeneration.
- Dunn *et al*.[6] have shown that the direct in vivo gene transmission of Ad (Adenoviral vector) / BMP-7 in a collagen gel carrier has facilitated the active regeneration of alveolar bone defects around dental implants. The study concluded that in-vivo gene therapy of BMP-7 provides promise for alveolar bone engineering applications.

Conclusion

Vectors in gene therapy is a revolutionary advancement in the field of genetics. The genetic basis of an individual is known to influence the severity and progression of periodontal disease.[28] The revolutionary discovery will open gates for better clinical outcomes in terms of understanding and treating severe periodontal diseases, which could benefit in treating genetic diseases associated in the field of dentistry .[29]

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